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NONPOINT SOURCE BACTERIAL POLLUTION IN THE COYOTE CREEK WATERSHED

A Thesis

Presented to

The Faculty of the Department of Environmental Studies

San José State University

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

by

Elisabeth Wilkinson

May 2018

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The Designated Thesis Committee Approves the Thesis Titled

NONPOINT SOURCE BACTERIAL POLLUTION IN THE COYOTE CREEK WATERSHED

by

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APPROVED FOR THE DEPARTMENT OF ENVIRONMENTAL STUDIES

SAN JOSÉ STATE UNIVERSITY

May 2018

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ABSTRACT

NONPOINT SOURCE BACTERIAL POLLUTION IN THE COYOTE CREEK WATERSHED

by Elisabeth Wilkinson

Nonpoint source pollution is a global challenge affecting streams and rivers. Due to the highly dispersed nature of nonpoint sources, they are difficult to track and manage. The goal of this study was to assess the extent of bacterial pollution along Coyote Creek in Northern California and to provide insight on microwatershed characteristics and their potential correlation to bacteria loads. Samples were collected and analyzed for fecal indicator bacteria concentrations at 21 geographic locations from within the creek and its stormwater outfalls. *E. coli* levels within Coyote Creek and stormwater entering the creek exceeded recreational water quality standards. Stormwater outfall samples tended to have higher bacteria concentrations than creek samples. The average concentration for stormwater outfall samples was 439% higher than the average for creek samples. Increased bacterial concentrations were found in creek samples draining adjacent to industrial microwatersheds when compared to samples draining residential microwatersheds. Bacteria levels from creek samples showed weak positive correlations with the number of historical encampments upstream of each sample site. This study concludes that bacterial load mitigation efforts should focus on industrial land uses and prioritize stormwater retention and treatment. The effects of homeless encampments on bacterial load may be less of a concern than other sources. Recommendations include further investigation of sources contributing to elevated bacteria levels exiting stormwater outfalls using DNA tracking methods.

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Motivation and Scope

Water pollution is an issue faced across the world in both marine and freshwater ecosystems. As the global human population increases, industrial and agricultural activities expand, degrading water quality. The greatest sources of water pollution are insufficient treatment of human waste and poorly managed industrial and agricultural waste (United Nations, 2009). As more water becomes polluted from anthropogenic activities, the supply of usable freshwater is depleted, negatively affecting drinking water, recreation activities, fisheries, and wildlife (United Nations, 2009). Globally, agriculture is the largest human use of freshwater, making up 70% of human water usage. Population growth is linked both to water used by humans for agriculture, domestic, and industrial uses and to pollutant emissions into waterways (de Sherbinin, Carr, Cassels, & Jiang, 2009; United Nations, 2009).

Water can become polluted from point and nonpoint sources. Point source pollution is easier to track than nonpoint source pollution, because the pollution enters the waterway through a physical pipe. Nonpoint source pollution occurs when rainfall or snowmelt moves over the ground and picks up pollutants, ultimately carrying them to rivers, lakes, oceans, and ground waters (Unites States Environmental Protection Agency, 2017). Nonpoint source pollution is especially difficult to prevent as it stems from multiple diffuse sources. Common nonpoint source pollutants include fertilizers, herbicides, insecticides, oil, toxic chemicals, sediment, bacteria, and salt (Unites States Environmental Protection Agency, 2017). Within the United States, nonpoint source

pollution is the primary remaining cause of water quality degradation (Unites States Environmental Protection Agency, 2017). Urban areas contribute to the amount of nonpoint source pollution ending up in waterways, as they contain more impervious surfaces. Impervious urban surfaces prevent rain from percolating into the ground, forcing water to travel over the surface before making its way into storm drains and eventually into streams, rivers, and the ocean (Unites States Environmental Protection Agency, 2017). Laws such as the Federal Clean Water Act help monitor and reduce water pollution in the United States.

Background

Under Section 303(d) of the Clean Water Act, impaired water bodies must be listed on the 303(d) list if they contain pollutants that exceed specific water quality standards and are not supporting their designated beneficial uses (Clean Water Act of 1972, 2011). Once a water body is placed on the 303(d) list, the appropriate Regional Water Quality Control Board evaluates the nature of the impairment and develops a Total Maximum Daily Load (TMDL). A TMDL is a plan that describes how an impaired water body will meet water quality standards. Thousands of waterbodies throughout California are listed as impaired for various pollutants, including many in the San Francisco Bay Area. Within the southernmost portion of Santa Clara County, the Pajaro River Watershed contains multiple creeks and rivers listed as impaired, including the Pajaro River itself (United States Environmental Protection Agency, 2014). The Pajaro River currently has TMDLs for nutrients, sediment, and fecal coliforms. The Pajaro River ultimately flows out to Monterey Bay. North of the Pajaro River Watershed is the Coyote Creek Watershed that

begins above Coyote Reservoir, flows through Anderson Reservoir, and continues north towards the San Francisco Bay through the cities of San Jose and Milpitas. Coyote Creek is currently 303(d) listed for trash impairments and has a TMDL for pesticides (United States Environmental Protection Agency, 2014). Although Coyote Creek is not 303(d) listed fecal coliforms, it has ongoing water quality problems and is vulnerable to fecal pollution from various anthropogenic sources.

Fecal coliforms are indicator organisms found in the intestines of warm-blooded animals that aid with digestion. Fecal coliforms are commonly used as an indicator that water has been contaminated with fecal waste because they are regularly excreted with fecal waste. Individuals exposed to water contaminated with fecal waste are at risk of contracting diseases such as gastroenteritis and hepatitis (Rose et al., 2001). Fecal waste can also be harmful to aquatic ecosystems, as aerobic decomposition of organic matter can reduce dissolved oxygen in waterways, harming fish and other aquatic organisms. Controlling fecal pollution to waterways is important for protecting both human and ecosystem health.

Creating management practices to prevent pollutants from entering waterways is important for the future health of river and marine ecosystems. Additionally, studying the relationships between land uses and specific pollutants, and investigating the specific sources producing the pollutants in the area, will help create and target best management practices.

Literature Review

Water is essential to the survival of living organisms, not only for supporting metabolism, but also for linking habitats together, as it serves as a vessel for transportation of sediments and nutrients. Water also provides a transportation pathway for pollutants to move through the environment (de Sherbinin et al., 2009). The transportation capacity of water is seen in the case of nonpoint source pollutants, which originate from multiple diffuse sources. As rainfall or snowmelt moves over the ground, it gathers pollutants and carries them through the landscape, where they are eventually deposited in fresh and coastal waters (United States Environmental Protection Agency, 2017). This long-distance travel can make nonpoint source pollution difficult to manage and track. Furthermore, it is especially difficult to control nonpoint source pollution using regulatory and socioeconomic tools, as cumulative effects of the pollution surpass political boundaries, and opportunities for polluters to "free ride" off the efforts of others persists (Lall, Heikkila, Brown, & Siegfried, 2008). For example, political boundaries can often reduce incentive to control or prevent nonpoint sources, as upstream areas may put effort into controlling sources, while the benefits of those efforts go to downstream areas in a different political zone (Lall et al., 2008). From a regulatory and enforcement standpoint, nonpoint source pollution has been more easily ignored compared to point source pollution, as it has a less directly visible effect on the environment. Cumulative impacts of nonpoint source pollution tend to surface in downstream areas, harming estuaries, bays, and wetlands (Lall et al., 2008). Craig and Roberts (2015) suggested that

the political will for regulation of nonpoint source pollution would be insufficient until water quality problems became apparent to the related community.

Cooperative Game Theory

Skardi, Afshar, and Solis (2012) used cooperative game theory as an alternative tool for analyzing land use and development and water-quality protection with regards to nonpoint source pollution. Cooperative game theory allows players to cooperate to improve their own payoffs. A hypothetical case study was employed and intended to have landowners control the total suspended solid outflow from their own respective catchments through wet pond construction, to satisfy a total target for sediment removal. Players participated in coalitions with one another if it provided them more benefits compared to their individual cost of satisfying the target. Skardi et al. (2012) assumed that because economic savings were possible through the cooperative game, players had enough incentive to participate in coalitions to achieve the total target for sediment removal. Furthermore, as the wet pond was the only available method to mitigate the sediment loads in this scenario, the best locations and sizes of wet ponds for minimum cost were able to be determined through watershed simulation models (Skardi et al., 2012).

Index Number Theory

Whittaker, Barnhart, Färe, and Grosskopf (2015) used the index number theory as a basis to obtain a single measure for water quality that encompassed information from multiple different water pollutants. Whittaker et al. (2015) emphasized that it is beneficial for stakeholders and policy makers to have a single measure of water quality when

discussing policy remediation. A water quality index was created using foundations from economic index theory for the Gulf of Mexico region in response to historical and ongoing Gulf hypoxia. Monthly water quality data from the United States Geological Survey between 1981 and 2013 were used to construct the index and included the following pollutants: dissolved nitrite, total Kjeldahl nitrogen, dissolved ammonia, total phosphorous, dissolved orthophosphate, and dissolved silica. Whittaker et al. (2015) concluded that the created water quality index accurately represented the aggregated characteristics of the various pollutants; however, it did not predict annual variation in the Gulf hypoxia.

Cultural Eutrophication

As humans alter natural landscapes through processes such as agriculture, waterways can suffer as a result. Eutrophication occurs when limiting nutrients in water bodies, usually nitrogen or phosphorous, increase, changing surface water quality in both freshwater and marine ecosystems (Smith, 2003). Eutrophication can lead to algal blooms and fish kills, as the increased nutrient loads stimulate the growth of aquatic plants and algae. Organic matter from fecal waste can reduce the dissolved oxygen supply as bacteria use it to metabolize organic matter. The large amounts of oxygen used by the bacteria result in less available oxygen for fish and other aquatic organisms. In severe cases, such blooms can lead to dead waterways with only anaerobic bacteria surviving (Schindler, 2012). Furthermore, eutrophication of fresh surface waters can lead to sea-bottom hypoxia in coastal ecosystems (Lall et al., 2008). Hypoxia occurs yearly in the northern portion of the Gulf of Mexico, where the nutrient-rich Mississippi River meets

the sea, creating the second largest hypoxic zone worldwide (Rabalais, Turner, & Wiseman, 2002). Eutrophication can be a natural process; however, when it is accelerated by increased nutrients from human activities, it is known as cultural eutrophication. Recognizing that human activities on land can negatively influence aquatic ecosystems is important for reducing human influenced water quality problems in natural ecosystems worldwide.

Pathogen Pollution

Another pollutant of concern for surface waters in the United States is pathogens. Pathogens are the leading cause of water impairment in the United States (United States Environmental Protection Agency, 2014). Pathogen presence is inferred from the presence of fecal indicator bacteria in water (Harmel, et al., 2016). Fecal indicator bacteria are found in the intestines of homeothermic animals, and they are excreted in fecal waste (Saxena, Bharagava, Kaithwas, & Raj, 2015). Fecal indicator bacteria are preferred over specific pathogen testing because of the extensive number of potential waterborne pathogens, time, and analytic expenses for pathogen testing (Harmel et al., 2016). Fecal indicator bacteria are also preferred because of their high levels in feces and their relationship to pathogen presence (Harwood, Staley, Badgley, Borges, & Korajkic, 2014). Fecal indicator bacteria are not harmful to human health themselves, but they are correlated with harmful pathogen presence in water. Common fecal indicator bacteria used to indicate pathogen presence in water include *Escherichia coli* (*E. coli*), and Enterococci (Al Bakri, Rahman, & Bowling, 2008; Bolstad & Swank, 1997; Avigliano & Schenone, 2015; Harmel et al., 2016; Hathaway, Hunt, & Simmons, 2010; Harwood et

al., 2014; Paule-Mercado, et al., 2016; Pettus, Foster, & Pan, 2015; Saxena et al., 2015; Stumpf, Piehler, Thompson, & Noble, 2007; Tran, Gin, & Ngo, 2015).

Health Effects

Microbial contamination of waterways caused by fecal pollution can have direct negative health effects for humans (Harmel et al., 2016). Humans are most at risk upon ingestion of water contaminated with human or other vertebrate feces (Cabral, 2010). Ingestion of water contaminated with feces of infected humans or animals can cause gastroenteritis, typhoid, bacillary dysentery, amoebic dysentery, cholera, meningoencephalitis, meningitis, and hepatitis (Saxena et al., 2015). In the United States, an estimated 560,000 people experience waterborne diseases and 7.1 million people incur mild to moderate infections, resulting in roughly 12,000 deaths per year (Cabral, 2010).

Besides direct intentional ingestion of contaminated water, recreational uses of water can also result in indirect, unintentional ingestion. This can pose a health risk, and limit the beneficial use of the contaminated waterway. In California, the Porter-Cologne Water Quality Control Act allows designation of specific beneficial uses to waterways in Basin Plans. A Basin Plan serves as a master policy document for water quality regulation in a specific region. The San Francisco Bay Basin Plan defines the water contact recreation beneficial use as "Uses of water for recreational activities involving body contact with water where ingestion of water is reasonably possible. These uses include but are not limited to, swimming, wading, water-skiing, skin and scuba diving, surfing, whitewater activities, fishing, and uses of natural hot springs" (California Regional Water Quality Control Board San Francisco Bay Region, 2017). The non-contact recreational beneficial

use is defined as, “Uses of water for recreational activities involving proximity to water, but not normally involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, picnicking, sunbathing, hiking, beachcombing, camping, boating, tidepool and marine life study, hunting, sightseeing, or aesthetic enjoyment in conjunction with the above activities” (California Regional Water Quality Control Board San Francisco Bay Region, 2017). The United States Environmental Protection Agency (US EPA), created the 2012 Recreational Water Quality Criteria for estimated illness rates of recreating in waters containing high bacteria levels. These criteria are provided to protect public health and help identify if surface water is meeting recreational beneficial uses. Enterococci and *E. coli* are the fecal indicators used to estimate illness rate in the criteria (United States Environmental Protection Agency, 2012).

Combined Sewer Systems

A combined sewer system collects rainwater runoff, domestic sewage, and industrial wastewater into the same pipe before transport to a treatment plant. Combined sewer overflow occurs when the sewer system exceeds its capacity during storm events and discharges extra wastewater directly to streams or rivers without treatment. Furuma, Nakajima, & Katayama (2011) found that combined sewer overflows are serious sources of pollution during rain events and that concentrations of human adenoviruses and *E. coli* were positively correlated near sewer overflows. Consideration of the type of sewer system present in urban areas is important as it can affect the risk of fecal contamination in waterways and associated human health problems, especially during storm events.

Water Quality Land Use Relationship

Urbanization and agriculture. The correlations of varying land uses on river water quality are documented throughout the literature (Bu, Meng, Zhang, & Wan, 2014; Kang et al., 2010; Paule-Mercado et al., 2016; Pettus et al., 2015; Qin, Khu, & Yu, 2010; Zhou, Huang, Pontius, & Hong, 2016). Multiple studies correlated different land use types with water quality (Bolstad & Swank, 1997; Donohue, McGarrigle, & Mills, 2006; Dodds & Oakes, 2008). Land uses associated with more anthropogenic activities, such as agriculture or urban, consistently have higher bacterial concentrations than more natural riparian landscapes (Kang et al., 2010; Pettus et al., 2015; Schoonover & Lockaby, 2006). Land cover development, rapid urbanization in developing countries, changes in land use management, and spatial land use patterns are also correlated to water quality conditions. Watersheds containing several different land use types are more likely to have poor water quality. Land cover conversions to bare land and configurations and proportions of land use type are closely associated with water quality (Paule, Memon, Lee, & Umer, 2014; Lee, Hwang, & Sung, 2009; Line, 2002; Qin et al., 2010; Alberti et al., 2007; Kwang-Wook, Sang-Woo, Ha-Sun, & Jae-Ho, 2008; Xiao & Ji, 2007). Furthermore, urbanized land use has been identified as a leading contributor to *E. coli* and Enterococci concentrations in waterways (Kang et al., 2010; Pettus et al., 2015; Schoonover & Lockaby, 2006).

Ding et al. (2016) considered the effects of low order stream water quality and land use relationships on downstream water quality in the highly urbanized Dongjiang River Basin in China. Low order streams are those that are smaller and transport water and

associated pollutants, nutrients, sediments, and organic materials to larger downstream river systems (Ding et al., 2016). Water temperature, dissolved oxygen, electrical conductivity, permanganate index, total phosphorus, total nitrogen, ammonia nitrogen, nitrate nitrogen, and chlorophyll were measured. Degraded water quality was associated with higher densities of agricultural lands in the mountain areas, and correlated with urban land use in the plains regions, suggesting that the spatial configuration of the land use types had the greatest effect on water quality (Ding et al., 2016). The investigation into low order streams in this study did not consider bacterial pollution as a variable, which leaves a gap within the literature of the effects of surrounding land use on bacteria in low order streams. Furthermore, data were collected during base flows leaving out valuable storm event data likely to reveal the first flush effect.

Permeability. Permeability is an important land characteristic that correlates to water quality within watersheds, and is associated with urban land uses and impervious surfaces (Mallin, Williams, Esham, & Lowe, 2000; Kang et al., 2010). Impervious surfaces such as roads, parking lots, roofs, and driveways prevent infiltration of stormwater on land and allow more polluted stormwater runoff to enter rivers. Mallin et al. (2000) found the percent of impervious surface area within estuarine watersheds explained 95% of the variability in estuarine fecal coliform abundance.

First Flush

The beginning of a storm, when the highest concentrations of pollutants are present and then decline as they are flushed out of the area, is considered the first flush (Stumpf et al., 2010). Pollutants can build up and accumulate on solid surfaces over time,

especially when there are extended temporal gaps between storm events. When the first storm event occurs, the accumulated pollutants are carried or "flushed" away by the water into streams, resulting in a spike in pollutant concentrations during the first storm event (Stumpf et al., 2010). The concept of a first flush has been used for studies concerning fecal indicator bacteria and land use relationships, as well as for other pollutant-water-quality land-use relationships. Furthermore, areas with more impervious surfaces tend to have more intense first flushes than areas with higher proportions of pervious surfaces, as they provide less filtration and absorption than pervious surfaces (Lee & Bang, 2000; Qin et al., 2010).

Point Sources

The effects of point source pollution on nonpoint source and land use relationships is a new realm of investigation for water quality and land use. Zhou et al. (2016) concluded that point sources of pollution greatly weaken the correlation between land use and water quality within a watershed, revealing that point source pollution may be masking the nonpoint source correlations (Zhou et al., 2016). This study underscores the importance of considering different pollution types and sources when studying how land use affects water quality.

Tracking Methods

Tracing fecal indicator bacteria to source organisms can help identify nonpoint sources of fecal pollution. Chemical markers are one method used to trace fecal bacteria to source organisms. Chemical markers can be especially useful for human-source specificity and require less lab analysis time than bacterial testing (Glassmeyer et al.,

2005). Sankararamakrishnan & Guo (2005) used chemical markers to indicate human fecal coliforms in stormwater outfalls. The chemical markers used in this study to indicate human waste contamination were caffeine, anionic surfactant, fluoride, and fluorescent whitening agent. The study was conducted at Deal Lake in New Jersey during dry weather, and at various sites around Deal Lake and directly at stormwater outfalls during wet conditions. Sankararamakrishnan & Guo (2005) found that concentrations of the markers were much higher during wet weather, than those tested in Deal Lake during dry weather, which indicated human waste presence at stormwater outfalls. Sankararamakrishnan Guo (2005) also found strong correlations between fecal coliform counts and all chemical marker values.

Fecal sterols are another chemical marker method used to trace fecal indicator bacteria to sources (Lyons et al., 2015; Fahrenfeld, Del Monaco, Coates, & Elzerman, 2016). Coprostanol is a chemical within fecal sterols produced in higher level mammal intestines during the digestion of cholesterol. Coprostanol accounts for between 40-60% of all fecal sterols in human fecal waste, (Fahrenfeld et.al, 2016). Lyons et al. (2015) analyzed fecal sterol for coprostanol to link sewage contamination to anthropogenic sources. Use of chemical marker fecal sterols are recommended over bacterial marker methods especially for marine environments, as fecal sterols do not degrade as quickly in marine environments (Lyons et al., 2015; Rozen & Belkin, 2001).

A more exotic method of fecal indicator bacteria tracking used canines trained for the scent of human waste. Van De Werfhorst, Murray, Reynolds, & Holden, (2014) found canine scent trackers were between 70-100% accurate at predicting where human waste

was present in storm drains. Findings were confirmed with fecal indicator bacteria and microbial source tracking methods. With further research, this method may be a viable option to track fecal indicator bacteria to human waste in waterways.

Microbial source tracking (MST) is a method used to identify the host species of fecal indicator bacteria from water samples. Microbial source tracking analyzes fecal indicator bacteria commonly found in the intestines of warm blooded animals that regularly get excreted with feces. Characteristics of fecal indicator bacteria are associated with specific hosts, which allows for identification of the host species (Harwood et al., 2014). Microbial source tracking is more accurate than chemical marker tracking because it uses known host-specific gene sequences from the DNA of microorganisms (Kim & Wuertz, 2015; Panasiuk, Hedström, Marsalek, Ashley, & Viklander, 2015). Microbial source tracking is the current method suggested to detect organism sources for fecal coliform and can distinguish between many different animal species (Panasiuk et.al, 2015). There are two main strategies of analysis used for microbial source tracking including library-dependent analysis and library-independent analysis. Library-dependent analysis includes the creation of a database of attributes from known fecal sources, made up of phenotypic or genotypic attributes of the fecal indicator bacteria specific to different hosts. The fecal indicator bacteria in the water sample are then classified by referencing the database (Ahmed et al., 2007; Graves & Hagedorn, 2010). The library-dependent method is limited by the size of the reference library and by the geographic and temporal variation in the fecal indicator bacteria specific to certain hosts (Badgley & Hagedorn, 2015). Library-independent methods do not require a reference database as they use the presence

of genetic markers associated with the fecal source. Quantitative polymerase chain reaction (qPCR) is the preferred method for detecting the genetic markers in fecal indicator bacteria because it can determine relative amounts of the material in the water sample rather than presence-absence values (Badgley & Hagedorn, 2015). Due to their abundance, the order Bacteroidales are prevalently used for qPCR MST studies for humans, livestock, and domestic animals (Kim & Wuertz, 2015). When using qPCR MST analysis, it is recommended to use multiple markers for identifying a single host species (Badgley & Hagedorn, 2015). Host associated markers can vary depending on the region, and it is recommended to have them locally validated. These differences can be attributed to diet differences of the hosts species and differences in decay rates of the indicators under varying environmental conditions. No single method of tracing fecal indicator bacteria to hosts offers efficient, precise, and inexpensive results (Panasiuk et al., 2015). Therefore, it is recommended that multiple methods are combined for the most accurate results.

Previous studies conducted using microbial source tracking offer insight for future studies. Ervin, Van de Werfhorst, Murrary, & Holden (2014) analyzed fecal indicator bacteria sources using MST in the Arroyo Burro Watershed of Santa Barbara, California. Surface waters, including surf zones and creeks leading to the beach, were sampled over two years for tracking sources of contamination in a suburban coastal beach. Dissolved oxygen, electrical conductivity, and temperature were measured at each site, along with creek and lagoon outlet water velocities, and channel dimensions for calculating flow rate. The result of the MST analysis revealed dog feces as an important contamination

source. Investigation of fecal indicator bacteria concentrations in conjunction with MST helped to target the source area. When the lagoon was discharging into the surf zone sample area, samples exceeded the California single sample limit. When the lagoon outlet was not flowing, surf zone samples were at or below the detection for *E. coli* and Enteroccoci. Dissolved oxygen and temperature were higher on average in the lagoon compared to the other surface water areas. An education and outreach program was implemented in surrounding areas regarding proper pet waste disposal, and resulted in decreased dog markers in downstream sample sites. Feces from coyote and fox were also analyzed as some sample areas had no homes with domestic dogs, and both coyote and fox feces had dog markers similar to domestic dogs under the MST analysis. Additionally, deer and raccoon feces had quantifiable dog markers. This showed multiple species may have contributed to the dog result found at the sites. Ervin et al. (2014) also found detectable levels of human markers in upstream areas that were reduced before reaching downstream locations, consistent with a previous study where the human marker decayed more rapidly than cultivated *E. coli* under environmental conditions (Dick, Stelzer, Bertke, Fong, & Stoeckel, 2010). MST markers have been inconsistent and not exclusive to single host species. For example the HF183 *bacteroides* marker used to identify human associated waste is occasionally found in dog feces (Bianco et al., 2015; Chase, Hunting, Staley, & Harwood, 2012).

Use of tracer dyes with MST studies may provide useful insight for deciding the best times to sample during precipitation events. Ohad et al. (2015) collected samples monthly for one year in four separate springs in Israel, three of which were one kilometer apart.

Daily samples were also taken during precipitation events and analyzed for ruminant, bovine, swine, and human sources. Human markers were found throughout the monthly samples year-round, which indicated continuous contamination from human sewage sources, rather than spikes from specific rain events. Markers peaked on the fifth day of the rain events. Ohad et al. (2015) found a connection between a previous study that took between 88 and 90 hours for a mixture of uranine (tracer dye) and LiCl placed fourteen kilometers away to reach the same three springs in close one kilometer proximity to each other, portraying a possible large contamination area contributing to the detected MST markers.

Different types of microorganisms can be used for MST (Bianco et al., 2015; Jofre, Blanch, Lucena, & Muniesa, 2014). Bianco et al. (2015) used both bacterial microorganisms as biological markers of certain species, as well as archaeal microbes. Archaea are a group of microorganisms separate from bacteria. Sample collection was split between dry and wet weather in the Sao Jao river basin of Rio de Janeiro. Archaea markers were more prevalent in the feces and water bodies than bacteria (Bianco et al., 2015). However, the same is not true for other studies (Gourmelon et al., 2007).

Jofre et al. (2014) analyzed the possibility of using bacteriophages as another type of tracker for MST. Bacteriophages are viruses that infect bacteria, and were found to be more abundant than bacteria in many environments. Bacteriophages that infect strains of Bacterioides have been detected in feces contaminated wastewater. Bacteriophages can only replicate inside their host and are more resistant to temperature, ultra violet (UV) radiation, and chemical disinfectants than common bacteria indicators. However, the use

of bacteriophages as a fecal source tracker is specific to the geographical area in which the samples are collected (Jofre et al., 2014).

Sediment

Sediment is an emerging factor to consider when analyzing streams for fecal pollution. Chase et al. (2012), conducted an MST study due to a fecal coliform impairment and subsequent TMDL for the New River that discharges into the Tampa Bay estuary in Florida. Much of the New River basin has soil with slow to very slow infiltration rates. The study assessed fecal sources by sampling water and sediment during flowing periods over eleven months on eight sample events. The study used the library-independent MST method for human and ruminant markers with PCR and qPCR, and collected water quality parameters including temperature, salinity, turbidity, pH, rainfall, flow rate, and dissolved oxygen. The water samples had significant positive correlations between *E. coli* and ionic strength, fecal coliforms and ionic strength, and *E. coli* and turbidity. Water and sediment samples had significant negative correlation between fecal coliforms and duration since the last rain. *E. coli* and fecal coliforms occurred in higher concentration during stagnant water conditions compared to flowing conditions, while enterococci concentration did not change due to flow conditions. Historical data also reflected this trend of lower *E. coli* and fecal coliforms during flow conditions, and it is attributed to their concentrations declining upon the large volumes of water added during flow events. During non-flowing conditions, the stream became isolated pools with large microbial occurrences. The use of enteroccoci was questionable, as multiple enteroccoci strains form naturalized resident populations in the

Tampa Bay watershed. Human sewage markers were found in all except for two of the seven sample sites at least once. These findings also occurred near known on-site sewage treatment and disposal systems. The quantities of detected human markers correlated with *E. coli* levels. However, the HF183 Bacteroides marker used to trace human sources is occasionally found in dogs. Ruminant markers were present in all except three sites. During the study the one-time sample maximum for recreational waters was exceeded in 100% of sampling events for enteroccoci, 62.5% for fecal coliforms, and 37.5% for *E. coli*. 100 grams of wet sediment also harbored 138, 88, and 36 times higher fecal indicator bacteria concentrations for *E. coli*, fecal coliforms, and enteroccoci respectively than those found in 100 ml of water at all sample sites. Chase et al. (2012) concluded that failing on-site sewage treatment and disposal systems and discharge from malfunctioning septic drain fields were the main sources of fecal bacteria in the New River.

Wilson, Schumacher, & Burken (2016) investigated *E. coli* sources that lead to multiple beach closings at Lake of the Ozarks State Park in Missouri. More than 1,100 samples of surface water, sediment, re-suspended sediment, and MST samples were collected. Other water quality parameters in the study included specific conductance, water temperature, pH, turbidity, and dissolved oxygen. Samples of re-suspended sediment, sediment, and water were used for MST and analyzed using qPCR. *E. coli* concentrations were significantly higher in the water column after mixing with the underlying sediments. Bather activity was cited as a primary component of spikes in *E. coli* concentrations due to the re-suspension and mobilization of the *E. coli* within the

sediment. Results were consistent with previous studies that cited longer survival time of *E. coli* in fine-grained sediments (Burton et al., 1987; Howell et al., 1995). Historical beach grooming was also a possible factor that explained the high amount of avian results as the grooming buried the fecal material and provided protection from sunlight, high temperatures, and drying. Wilson et al. (2016) also found high concentrations of human tracers in pools of runoff from an excavated area containing a two-year abandoned septic system.

Surbeck, Shields, and Cooper (2016) analyzed the effect of velocities on the transport of *E. coli* laden sediment in sand-bed streams and analyzed how sandy streambeds could be their own source of fecal indicator bacteria. Highest concentration of total coliforms occurred in downstream samples due to the transport of the coliforms and sand within the bed into the water column. Surbeck et al. (2016) suggested that sand-bed streams should be considered a source in the development of TMDLs.

Bradshaw et al. (2016) found that stream sediment was source of fecal indicator bacteria in surface water when physically disturbed, and indicated that re-suspension of sediment during storm events could be another source of fecal contamination to water. Bradshaw et al. (2016) also found that fecal indicator bacteria and MST marker concentrations from sediment samples were related to the pathogens analyzed in the water column.

Problem Statement

Coyote Creek is a reservoir-regulated stream within the Coyote Creek Watershed, which is the largest watershed in the Santa Clara Basin in Santa Clara County, California.

Coyote Creek is listed on the 303(d) list of impaired waterbodies for trash and the pesticide Diazinon. Although Coyote Creek is not listed for bacterial pollution, it is an ongoing water quality impairment. In 2015, San Francisco Baykeeper sued the City of San Jose for Clean Water Act violations pertaining to trash reduction requirements in their Municipal Separate Storm Sewer System Stormwater Permit and sewage discharge violations. As part of a consent decree, the City of San Jose must spend $100 million over ten years to rectify these issues (*Baykeeper v. City of San Jose,* 2016). Coyote Creek is an important habitat for the Santa Clara Basin's native fish species including Pacific lamprey, Central Coast Steelhead Trout and Sacramento Blackfish. Bacterial pollution in the creek not only affects wildlife habitat, but can also cause human health hazards in recreational situations where contact can occur.

To produce effective bacterial pollution control measures in Coyote Creek, the sources of the pollution and how they move through the modern pathways of stormwater need to be understood. Correlations between land uses, infrastructure, and fecal indicator bacteria will be useful to identify source areas of fecal pollution within the watershed that could benefit from best management practices This research will focus on correlations between various land use and infrastructure parameters and fecal indicator bacteria in Coyote Creek.

Research Questions and Hypotheses

Null hypotheses:

$H0_1$: Percent total m^2 impervious surfaces in a microwatershed will not correlate significantly with fecal coliform concentrations at outfalls.

$H0_2$: *E. coli* concentrations directly exiting stormwater outfalls will not differ significantly from *E. coli* concentrations in stream.

$H0_3$: Proximity to homeless encampments will not correlate with fecal coliform concentrations in the creek.

$H0_4$: Extent of sanitary sewer systems in a microwatershed will not correlate significantly with fecal coliform concentrations at outfalls.

$H0_5$: Population density of each microwatershed will not correlate significantly with fecal coliform concentrations at outfalls.

$H0_6$: Land use type of the microwatershed will not correlate significantly with fecal coliform concentrations at outfalls or in the creek.

Methods

Study Site

This study was conducted along Coyote Creek within the Coyote Creek Watershed in Santa Clara County, California. Coyote Creek drains an 860 km^2 watershed that extends from the western side of the Diablo Range into the valley where it covers a wide and heavily urbanized area North of Metcalf road before extending to the southernmost portion of the San Francisco Bay. Portions of the cities of Morgan Hill, San Jose, and Milpitas are within the watershed. San Jose is the largest city in the Coyote Watershed with a population of over one million. Forty-two creeks encompass the Coyote Watershed, with Coyote Creek the longest at 102 kilometers. The four major tributaries of Coyote Creek include Upper Penitencia Creek, Upper Silver Creek, Lower Silver Creek, and Fisher Creek. Coyote Creek flows through vastly different environments

throughout Santa Clara County. Coyote Creek's headwaters form near Henry W. Coe State Park in the Diablo Range east of Morgan Hill. The creek flows into Coyote Reservoir, and then north into Anderson Reservoir. After exiting Anderson Reservoir, the creek flows through unincorporated agricultural land between Morgan Hill and San Jose. Finally, Coyote Creek flows through the highly urbanized area of San Jose and into Alviso Slough before meeting the southern San Francisco Bay.

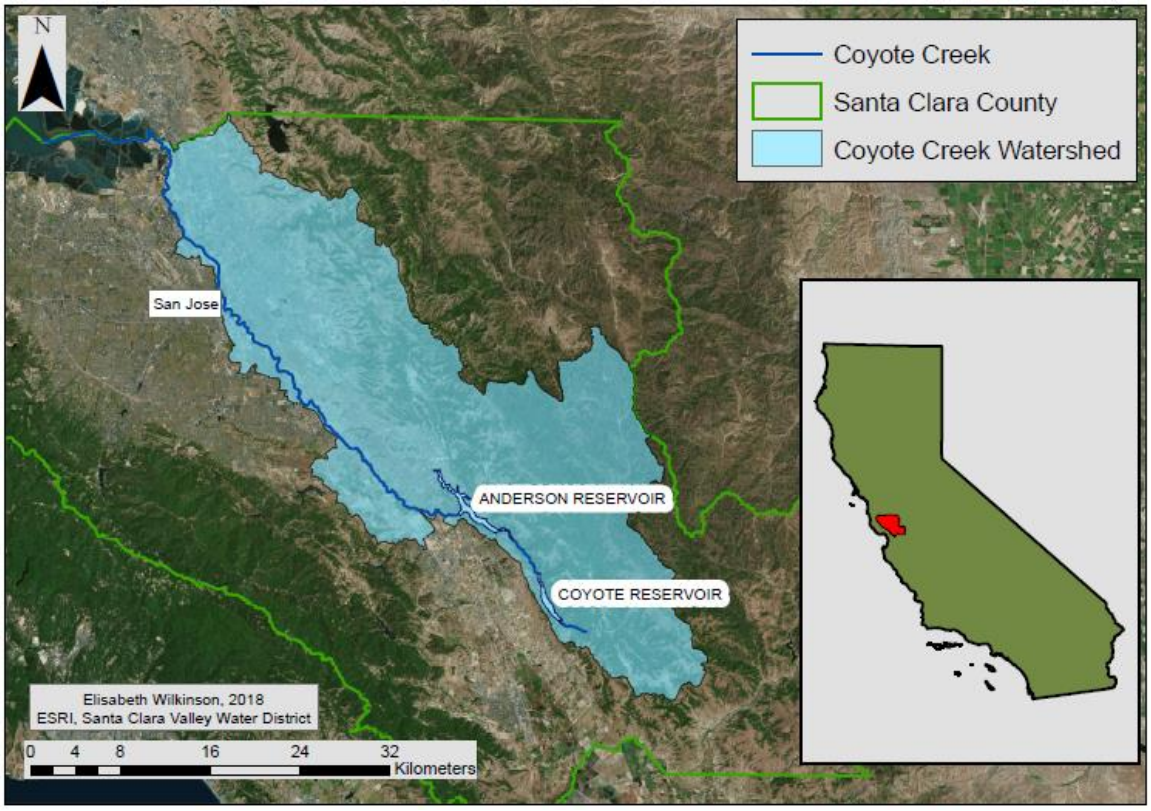

Figure 1. Location of Coyote Creek Watershed

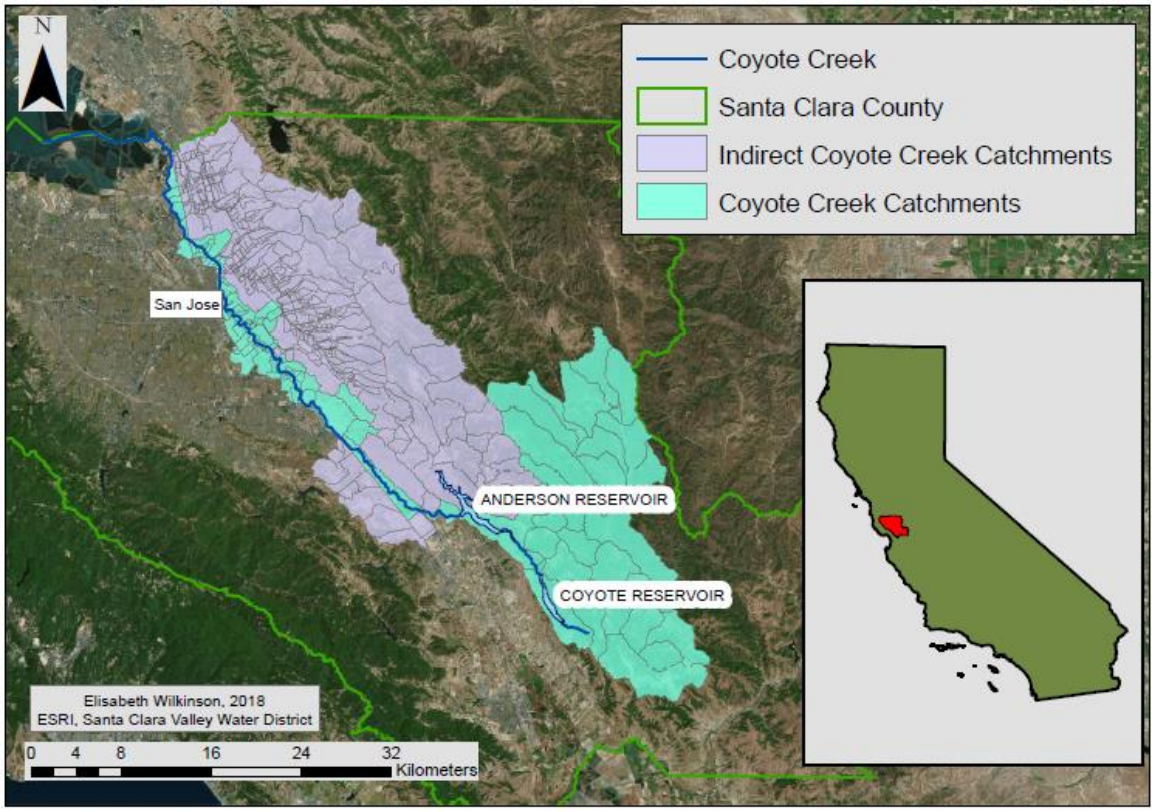

Figure 2. Coyote Creek Watershed Stormwater Catchments

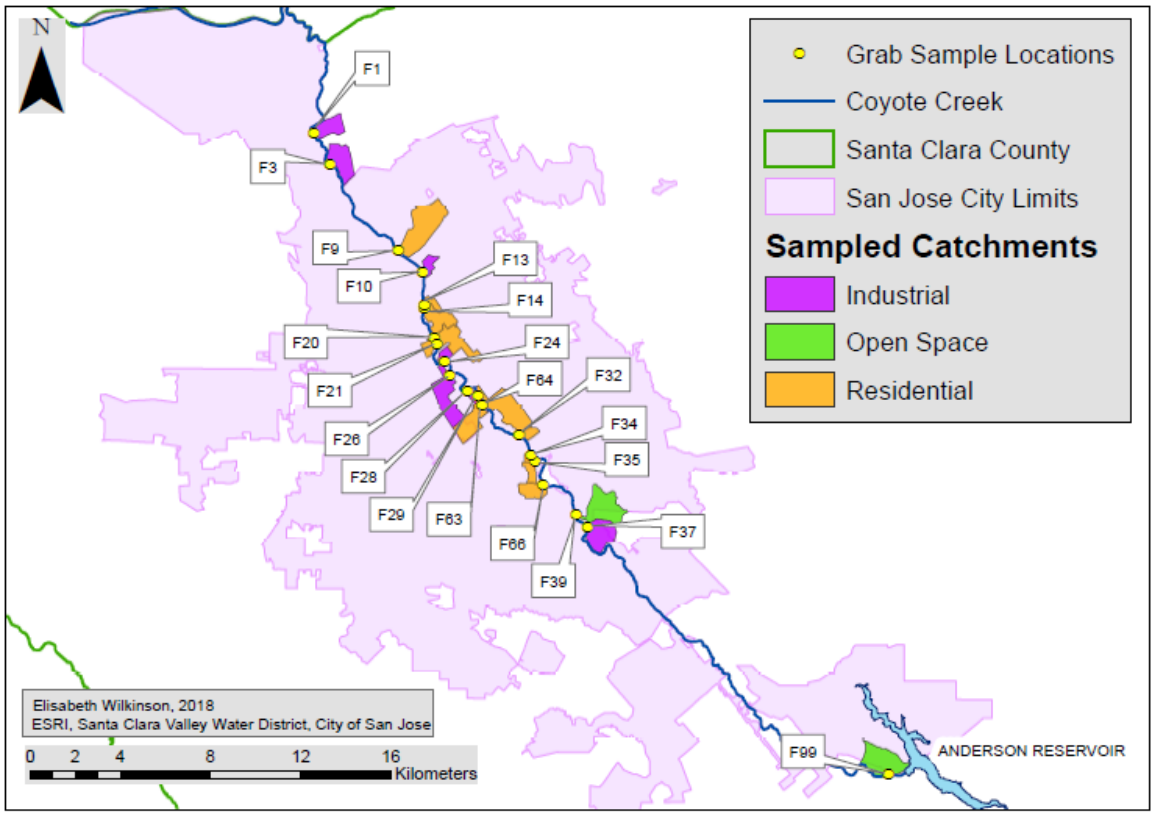

Figure 3. Coyote Creek Sample Locations and Identification

Study Design

Data used to assess the hypotheses included both secondary and primary data. Primary data were collected on November 3rd, 4th, and 9th of 2017 during wet weather events. The sampling units were microwatersheds of stormwater catchments provided as secondary data in shapefile format by the Santa Clara Valley Water District's HMP Catchments downloaded from their public GIS data download website. For primary data, grab samples were collected from water exiting stormwater outfalls as well as from the creek in urbanized areas in San Jose, and only from the creek around natural areas in the upper watershed that lack stormwater infrastructure.

Microwatersheds. Microwatersheds were chosen for the study because they provide a small geographic area with known boundaries and a single outlet that drains all water in the microwatershed. Microwatersheds were chosen in a stratified random manner that drain directly to Coyote Creek below Anderson Reservoir. The secondary data provided disclose what creek catchments drain to, but this was also confirmed by looking at the City of San Jose's storm sewer system network in ArcGIS. Dominant land use was also used to stratify sampling effort with major land uses of residential and industrial. Microwatersheds were then narrowed to areas on public property accessible by foot. A total of 21 microwatersheds were sampled at the outfalls that directly drain them, as well as from the creek adjacent to them. For all aspects of this study, the dependent variable was the concentration of *E. coli* in CFU/ ml in each collected sample.

Limitations. This study sampled outfalls and areas of Coyote Creek below Anderson Reservoir where the creek is dammed, and did not account for more rural catchments in the upper watershed above Anderson Reservoir. However, previous research has shown that rural areas in upper watersheds with less human influence often have better water quality than more urbanized areas (Kang et al., 2010; Pettus et al., 2015; Schoonover & Lockaby, 2006). Multiple chosen outfall sites were not flowing during rain events upon arrival to the site, and were removed from the study. Furthermore, sampled locations were limited to areas on public property that were accessible by foot, and determined safe to access while in the field. The sample size of the study was smaller than anticipated due to these factors, as well as the time and labor required to prepare, collect, and analyze

field samples. Generalization of results from this study are limited to watersheds with similar geography, land use, population density, and climate.

Data Collection

Primary Data. Grab samples are frequently taken in studies involving bacteria in water and land use correlations (Line, 2002; Lyons et al., 2015). Field procedures followed the National Pollutant Discharge Elimination System Stormwater Sampling Guidance Document (United States Environmental Protection Agency, 1992). Creek grab samples and water quality measurements were collected upstream of outfall samples to assess ambient water quality conditions without the influence of mixing from outfall contributions to the creek. Sampling equipment used included Nasco whirlpack sample bags, Garmin GPSMAP 64s unit, cooler, ice, gloves, and a Nasco whirlpack sampling pole. Water quality parameters were collected from the creek and at outfalls using an EXO2 multiparameter sonde. The sonde was placed directly in creek and outfall flow to collect measurements. When outfall flow was not deep enough to submerge the sonde, outfall water was collected in a one foot tall, black, cylindrical, bucket. The sonde was then placed in the bucket to collect measurements. After collection, grab samples were kept on ice in a cooler until analysis within 24 hours.

Secondary Data. Secondary data used for geographic information systems analysis was obtained from multiple public sources. Shapefiles for Coyote Creek and catchment areas were obtained from the Santa Clara Valley Water District online open data platform (2017). Shapefiles of stormwater outfalls, sanitary sewer lines, parcels, and zoning designations were downloaded from the City of San Jose's online data download (2017).

Zoning and parcel shapefiles were also downloaded from the City of Milpitas online data download (2017). Percent impervious data were obtained from the 2011 National Land Cover Database, downloaded from the USGS National Map Viewer online download site (2017). Homeless encampment cleanup frequency and location data were obtained from the City of San Jose and the Santa Clara Valley Water District through requests made under the Public Records Act.

Data Analysis

Enumeration of *E. coli*. All water samples were analyzed for *E. coli* using the spread plate method on MacConkey Agar II with MUG from Hardy Diagnostics. The agar was prepared in the microbiology lab at San Jose State University ahead of sample collection dates. Twenty-five grams of dried agar was mixed with 500 milliliters of millipore water in a 1000 milliliter beaker and then sterilized in an autoclave at 121 ˚C for 15 minutes at 15psi. After sterilization, the agar was placed into a water bath to cool for thirty minutes, and then poured into petri dishes. Once cooled, the agar plates were refrigerated in the Environmental Studies lab until use.

To accurately assess the level of bacteria in each grab sample, dilutions of 10^{-1}, 10^{-2}, and no dilution were plated using the spread plate method. All samples were vigorously shaken before pipetting and between pipetting for serial dilutions to ensure uniformity. All materials used for plating were sterile, and aseptic techniques were used to avoid contamination during plating. After plating petri dishes were incubated for 24 hours at 35 °C. Following incubation, pink, fluorescent *E. coli* colonies were identified using a longwave ultraviolet light. The number of colony forming units per milliliter was

calculated based on the number of colonies counted, the dilution number, and the amount of water plated.

Null hypothesis 1. To evaluate null hypothesis 1, the primary data, percent impervious surface data from the 2011 National Land Cover Database, and the HMP catchments from the Santa Clara Valley Water District were used. The zonal statistics tool in ArcGIS was used to obtain the average percentage of impervious surface within each microwatershed. Results of the zonal statistics tool were exported to Microsoft Excel and the primary data of *E.coli* in CFU/ml were entered into the spreadsheet for each associated microwatershed location. The Microsoft Excel sheet was then imported to IBM SPSS Statistics 24.0. All data were transformed using ln(x+1) and tested for normality in IBM SPSS with the Shapiro-Wilk test.

Null hypothesis 2. To evaluate null hypothesis 2, primary data in CFU/ml were ln(x+1) transformed for normality. Using the Shapiro-Wilk test in IBM SPSS, transformed data were considered normal at $p=.023$ ($p>.01$). A one-way ANOVA was then used to compare CFU/ml values in the creek versus at outfalls.

Null hypothesis 3. To evaluate null hypothesis 3, homeless encampment cleanup data from the Santa Clara Valley Water District and City of San Jose was used. The secondary data provided was in Microsoft Excel format and included the date and nearest street crossing description of encampment cleanup locations. Each cleanup location was separated by date in Microsoft Excel and street crossing descriptions were mapped in Google Earth. The number of times each encampment location was cleaned between November 2015 and November 2017 was also calculated from Microsoft Excel. The

measurement tool in Google Earth was used to obtain the distance of each outfall to the nearest upstream encampment. The number of total encampment cleanup sites upstream of each outfall was also obtained through Google Earth. Data were then imported to IBM SPSS and analyzed using simple linear regressions. Encampment locations cleaned between 2015 and 2017 were considered as "historical encampments" for this study, as the same locations tend to be consistently used as encampments based on the frequency of cleanup.

Null hypothesis 4. To evaluate null hypothesis 4, the shapefile of sanitary sewer system lines from the City of San Jose were clipped to the microwatersheds in ArcGIS. The tabulate intersection tool was used to calculate lengths of sewer lines in each microwatershed. The length of sanitary sewer systems in each microwatershed were then imported to IBM SPSS with the primary data. Simple linear regression was used to compare the length of sanitary sewer systems in each microwatershed to the *E. coli* in CFU/ml collected at the outfall and in the creek.

Null hypothesis 5. To evaluate null hypothesis 5, Population data from the American Community Survey were used to estimate population density of each microwatershed. The population data were downloaded at the block group level and imported into ArcGIS. Block group population data were then clipped to each microwatershed. The tabulate intersection tool was then used with the sum fields parameter to gain a population sum for each microwatershed based on the percentage of the block group intersecting the microwatershed zone. This provided only an estimate of population for each microwatershed. Block groups that were not entirely inside of a microwatershed

boundary had their population split based on the percentage of the block group that was within the microwatershed. This assumed that population is evenly distributed within a block group, which may not be true. After population estimates were obtained for each microwatershed, they were imported to IBM SPSS and divided by the area of the microwatershed to obtain an approximate population density. The population density and *E. coli* concentrations in CFU/ml were then analyzed using simple linear regression.

Null hypothesis 6. To evaluate null hypothesis 6, land use of each microwatershed was determined by using the tabulate intersection tool in GIS to calculate the percentage of each zoning type found in each microwatershed. Microwatersheds zoned 50% or greater of a single type were categorized under that land use. These land use designations were also verified using the most recent Google Earth satellite imagery. Final land use designations for sampled watersheds included residential, industrial, and open space. Residential and industrial results were included in the statistical analysis. Only one open space microwatershed was sampled, and therefore was not included in the statistical analysis.

Results

General Results

E. coli concentrations of samples collected from Coyote Creek and outfalls varied greatly (Figure 4). The average *E.coli* concentration was 403,765 cfu/100 ml for outfall samples, and 74,876 cfu/100 ml for creek samples. *E. coli* concentrations ranged between 0 and 630,000 cfu/100 ml for creek samples (Figure 5) and 0 and 4,600,000 cfu/100 ml for outfall samples (Figure 6). Although only single samples were taken at each sample

site, and geometric means cannot be calculated from single samples, values greatly exceeded the geometric means of the 2012 Recreational Water Quality Criteria. The criteria are calculated from a thirty-day geometric mean of 126 cfu/100 ml of water based on an estimated illness rate of 36/1000 (United States Environmental Protection Agency, 2012) The criteria state that no more than 10% of samples collected within the thirty-day window can exceed 410 cfu/100 ml *E.coli* (United States Environmental Protection Agency, 2012). Ninety percent of creek samples and 100% of outfall samples collected during this study exceeded 410 cfu/100 ml *E. coli*. Water quality parameters collected from the creek (Table 1) and outfalls (Table 2) during sample collection included temperature, conductivity, dissolved oxygen, total dissolved solids, turbidity and chlorophyll. Turbidity was positively correlated with *E. coli* concentrations (p=.023). This finding is consistent with previous research regarding significant positive correlations between *E. coli* and turbidity in surface water (Chase et al., 2012; Wilson et al., 2016). No other water quality parameters were found to have significant correlations with *E. coli* concentrations in this study.

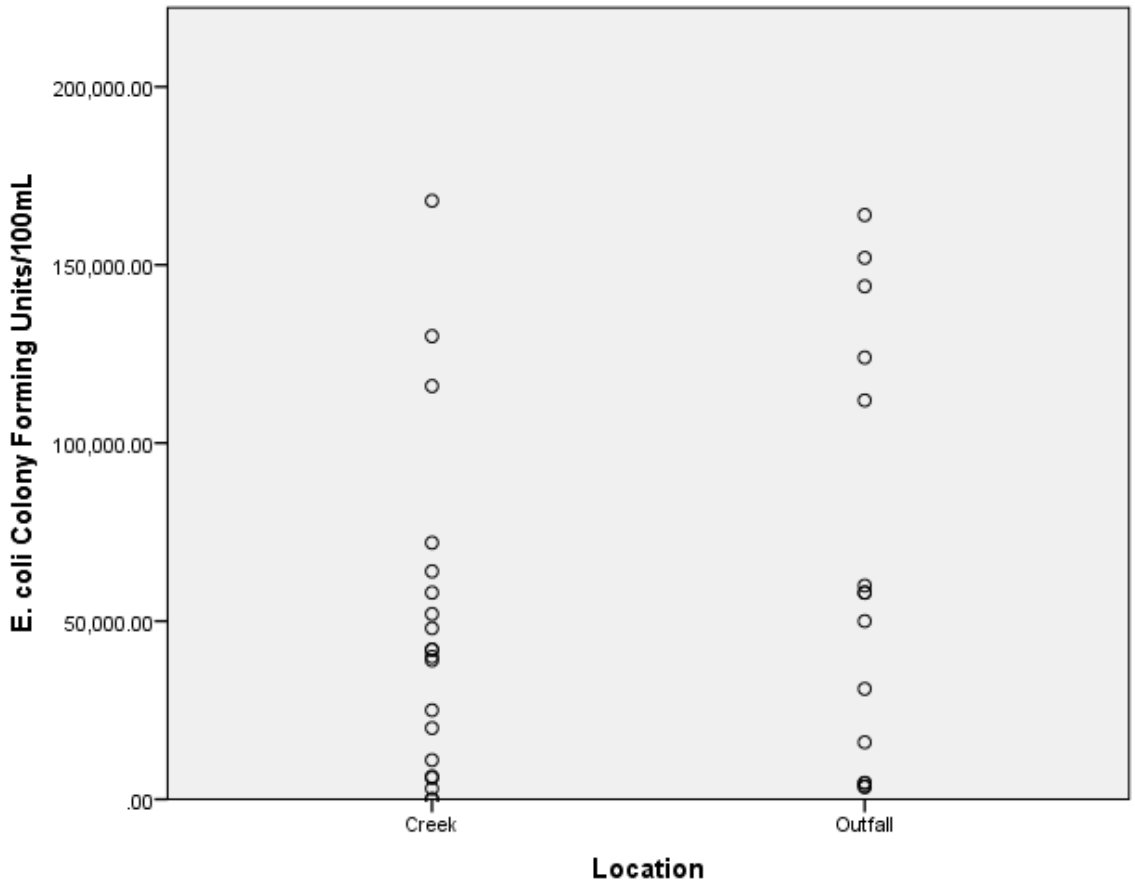

Figure 4. Creek and outfall E. coli results

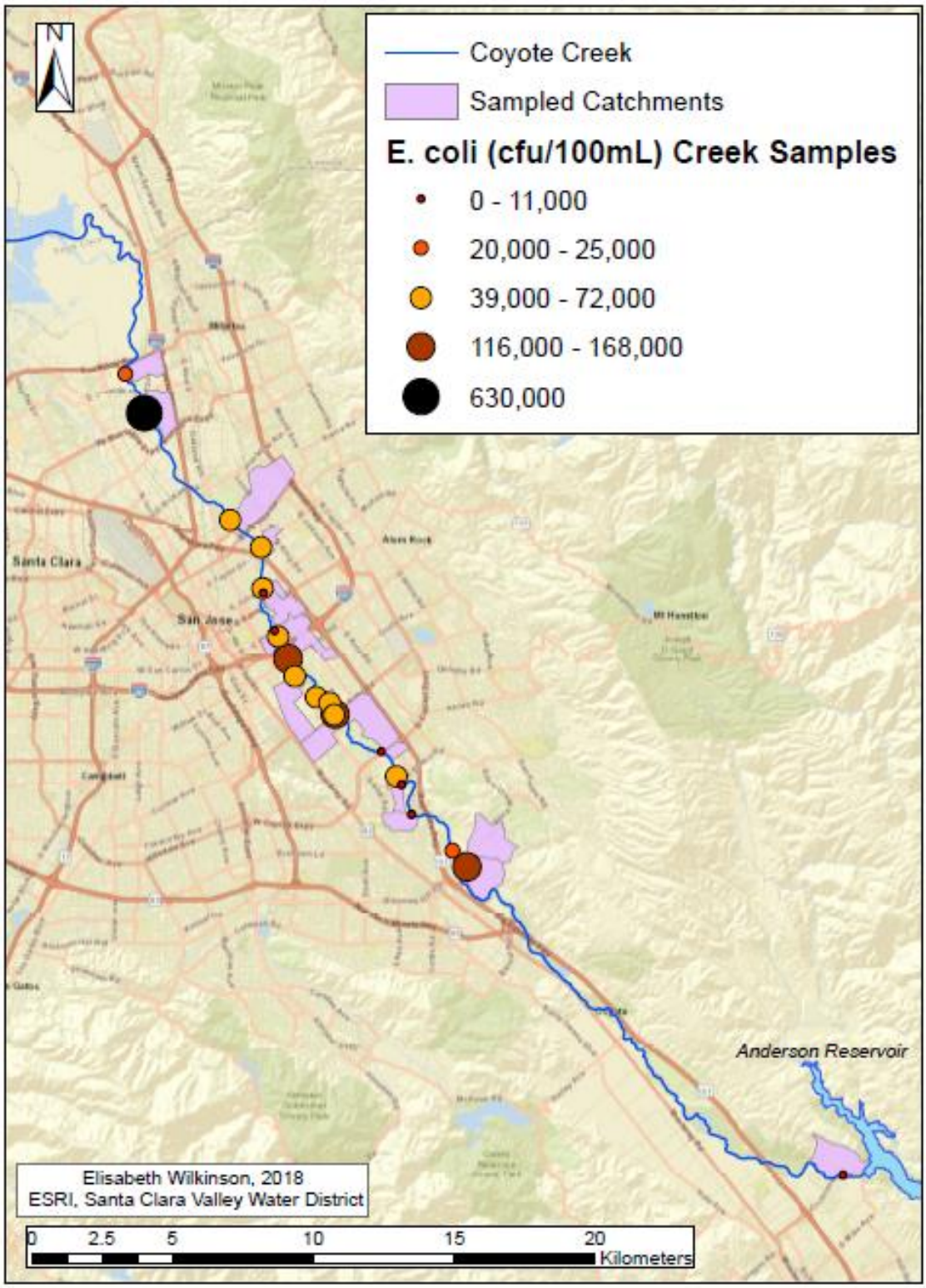

Figure 5. Map of E. coli results from creek samples

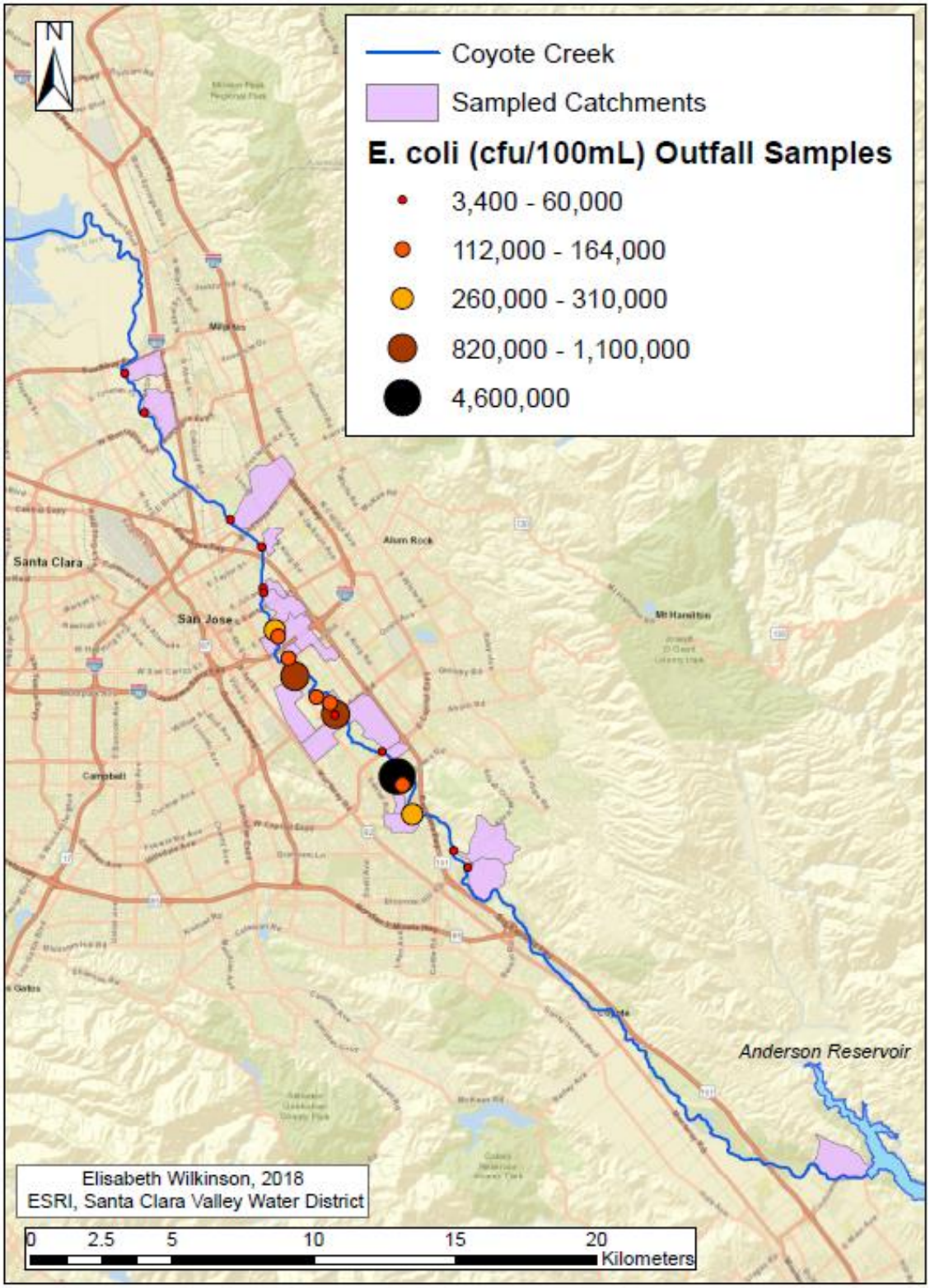

Figure 6. Map of E. coli results from outfall samples

Table 1.

Water Quality Results from Creek Samples

Site	Temperature (Celsius)	Conductivity (us/cm)	Dissolved Oxygen (mg/L)	Total Dissolved Solids (mg/L)	Turbidity (NTU)	Chlorophyll (µg/L)
F1	14.95	867.8	8.44	698	16.91	1.15
F3	15.13	885.6	8.43	709	15.55	1.2
F9	17.82	943.1	9.09	710	5.94	0.93
F10	14.76	835.8	7.87	675	17.22	2.05
F13	12.99	671.2	7.27	566	24	2.16
F14	14.71	660.6	6.8	534	10.13	2.74
F20	14.93	683.6	7.65	550	18.69	2.46
F21	14.75	652.7	8.34	528	68.04	2.91
F24	15.31	624.3	8.13	498	13.69	3.36
F26	14.84	613.5	8.05	495	12.37	2.36
F28	15.15	597.6	9.43	479	11.01	1.86
F29	14.54	536.5	8.77	436	10.14	2.8
F63	13.08	508.7	8.74	428	11.64	2.11
F64	13.15	507.9	8.82	427	24.56	3.73
F32	14.67	521.2	9.9	422	8.57	3.06
F34	14.03	464.7	9.7	382	25.26	3.54
F35	13.36	477.3	9.19	399	7.84	1.93
F66	13.95	485.8	9.77	400	4.53	1.63
F39	14.97	451.4	9.82	363	13.72	3.91
F37	15.35	434	10.22	346	5.24	3.24
F99	15.90	344.2	9.63	271	2.8	1.06

Table 2.

Water Quality Results from Outfall Samples

Site	Temperature (Celsius)	Conductivity (us/cm)	Dissolved Oxygen (mg/L)	Total Dissolved Solids (mg/L)	Turbidity (NTU)	Chlorophyll (µg/L)
F1	16.05	1404.6	7.81	1101	3.7	7.86
F3	14.72	848.1	7.3	686	6.1	6.44
F9	20.06	1270.2	9.31	912	1.61	0.17
F10	19.77	951.6	8.54	687	342.51	0.33
F13	16.23	422.3	8.32	330	11.63	9.67
F14	18.51	1641	8.44	1217	1.68	0.88
F20	18.06	865.7	2.49	649	122	8.66
F21	19.17	590.2	7.94	432	264.76	2.22
F24	17.40	266.3	9.63	202	60.69	17.37
F26	19.38	741.3	6.51	540	143.11	14.97
F28	17.04	286.8	8.37	220	26.37	25.12
F29	16.76	420.2	7.32	324	44.67	24.27
F63	16.94	574.7	7.57	442	28.69	7.82
F64	16.25	590.9	3.94	461	88.43	5.63
F32	15.14	398.2	6.68	319	80.67	17.13
F34	18.00	175.9	8.77	132	91.3	8.04
F35	18.27	196.1	8.73	146	76.2	9.02
F66	17.73	112.1	9.28	85	186.12	6.2
F39	18.07	731.8	9.58	548	13.62	8.85
F37	16.22	151.5	9.29	118	24.93	13.5

Hypothesis 1

Percent impervious surface of each microwatershed did not change with *E. coli* concentrations at outfalls, adjusted R^2= -.054, F(1,17)=.079, p=.783.

Hypothesis 2

E. coli concentrations exiting stormwater outfalls did not differ from *E. coli* concentrations collected from the creek, adjusted R^2= .058, F(1,41)=3.475, p=.070 (Figure 7). However, outfall samples tended to have higher mean *E. coli* concentrations than creek samples (Figure 8).

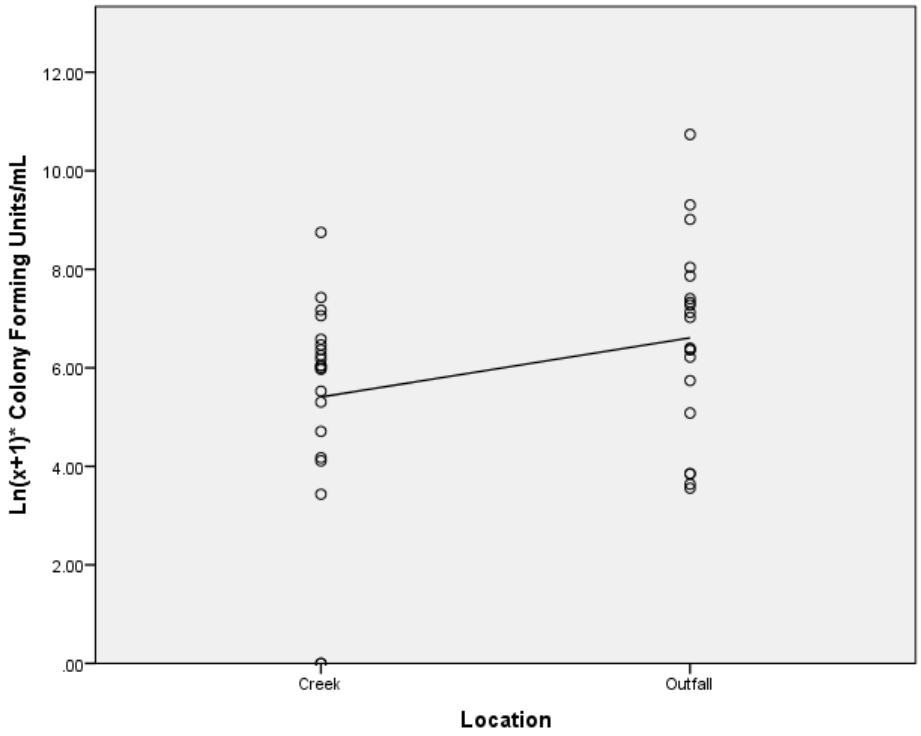

Figure 7. Creek and outfall E. coli concentrations

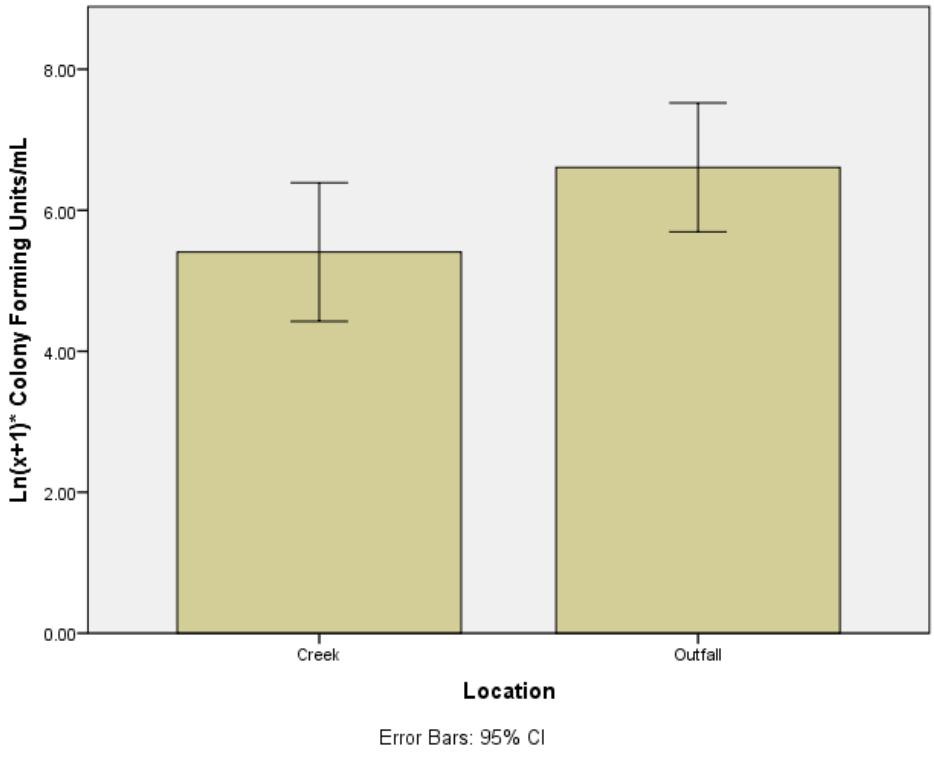

Figure 8: Bar chart of creek and outfall E. coli results

Hypothesis 3

Proximity to nearest upstream encampment site did not change with the concentration of *E. coli* in creek samples, adjusted R^2= .010, F(1,14)=1.158, p=.300. The number of times the nearest upstream historical encampment had been cleaned between 2015 and 2017 did not change with the concentration of *E. coli* in creek samples, adjusted R^2= .071, F(1,19)=2.539, p=.128. Additionally, the number of total historical encampment sites upstream of each creek sample location showed a weak positive trend with the concentration of *E. coli* in creek samples. adjusted R^2= .128, F(1,19)=3.935, p=.062 (Figure 9).

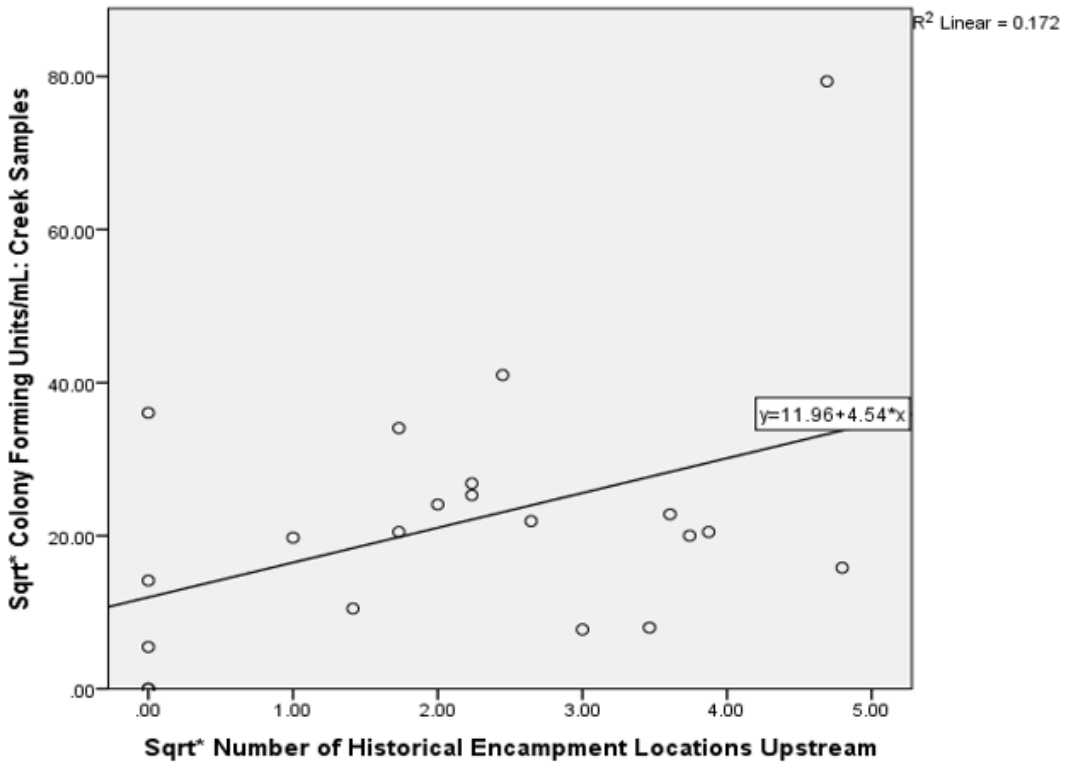

Figure 9. Creek and encampment locations E. coli results

Hypothesis 4

Extent of sanitary sewer system infrastructure within each microwatershed did not change with *E. coli* concentrations at outfalls, adjusted R^2= -.040, F(1,18)=.264, p=.614.

Hypothesis 5

Population density of each microwatershed did not change with *E. coli* concentrations at outfalls, adjusted R^2= -.005, F(1,17)=.914, p=.353.

Hypothesis 6

E. coli concentrations at outfalls did not change when compared to the land use of the microwatershed, adjusted R^2=.020, F(1,18)=1.366, p=.259 (Figure 10).

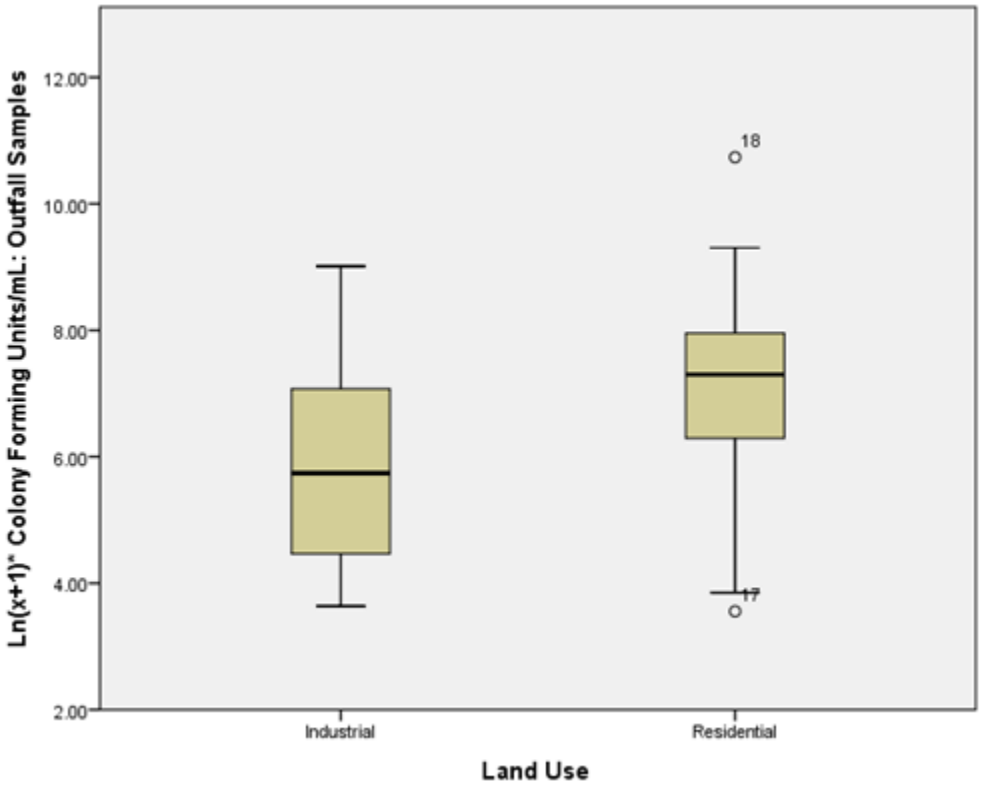

Figure 10. Industrial and residential microwatersheds and outfall E. coli concentrations

E. coli concentrations from the creek were higher when adjacent to industrial microwatersheds compared to residential microwatersheds, R^2=.287, F(1,18)=6.839, p=.018 (Figure 11, Figure 12). While not included in the statistical analysis, the concentration in cfu/ ml of the creek sample collected from an open space land use microwatershed, was lower than concentrations found in other land uses (Figure 13).

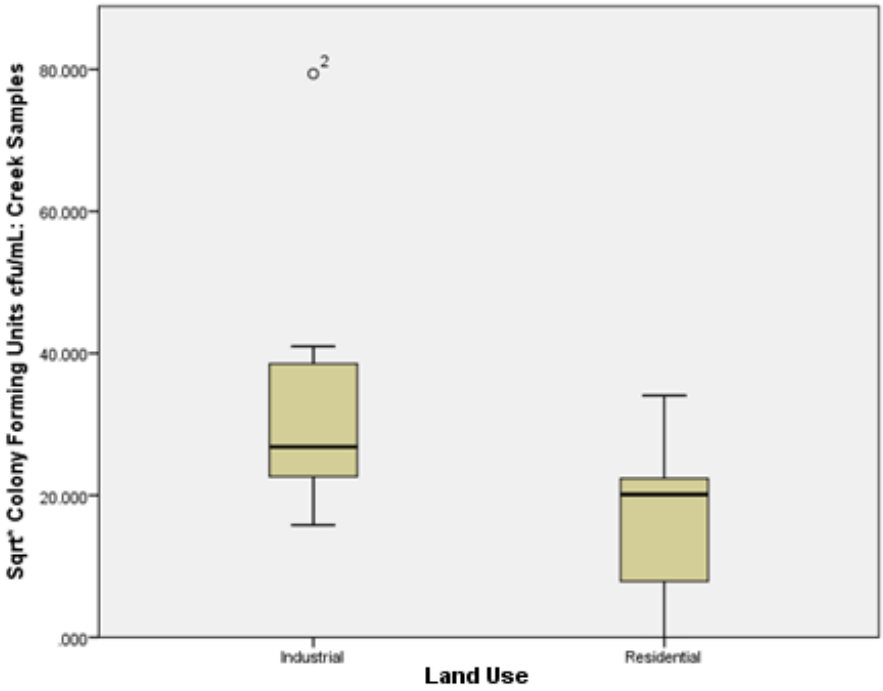

Figure 11. Box plot of industrial vs residential microwatersheds and creek E. coli concentrations

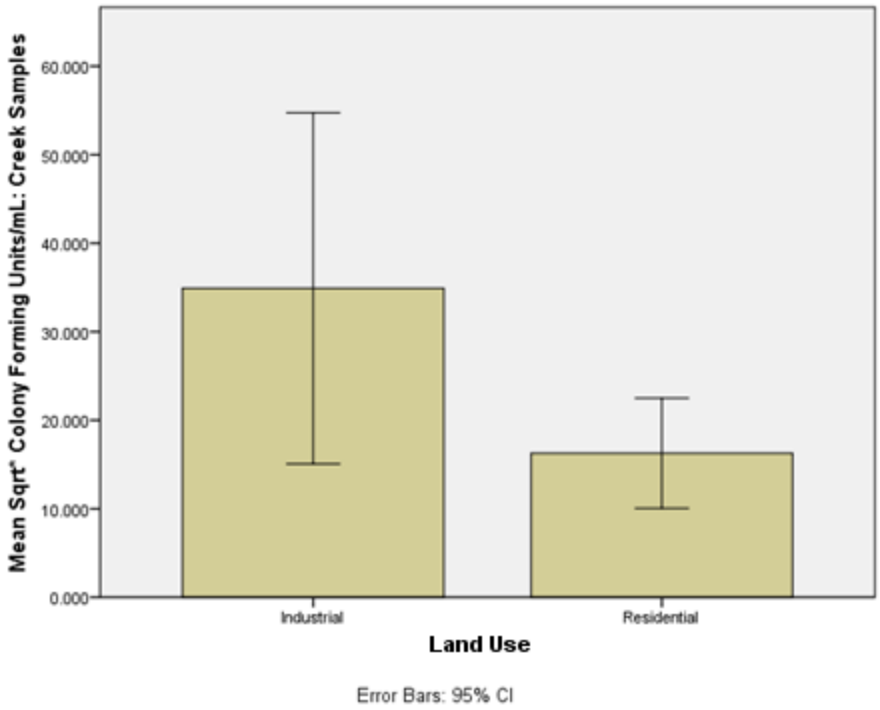

Figure 12. Bar chart of industrial vs residential microwatersheds and creek E. coli concentrations

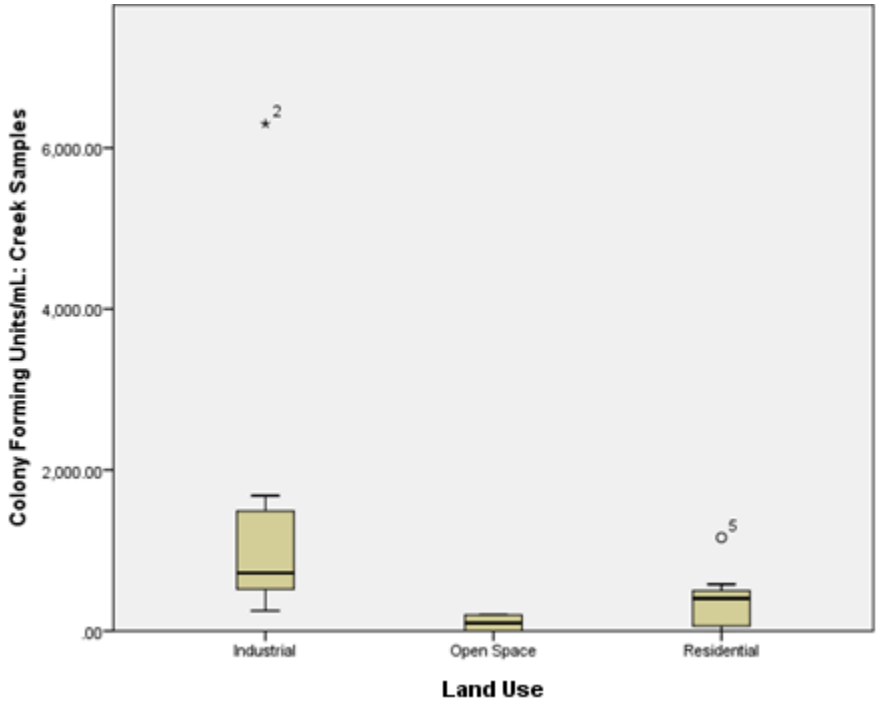

Figure 13. Box plot of industrial, residential, and open space land use and creek E. coli concentrations

Discussion

Results of this study were not consistent with previous studies concerning percent imperviousness of a watershed and water quality. In previous studies, percent impervious area of catchments was highly correlated with water quality, which was not found to be significant in this study (Mallin, et al., 2000; Kang et al., 2010). Results are also not consistent with previous studies that have correlated more populated areas to water pollution. Possible reasons for these inconsistent results may be that the analysis was conducted on small, specific microwatersheds that directly drained to Coyote Creek, rather than the entire Coyote Creek watershed and its tributary streams. Furthermore, the sample size was small compared to previous studies, and thus may not have been able to reveal these concepts. Extent of sewer systems also did not change with *E. coli* concentrations at outfalls. However, the presence of leaking sewer infrastructure or illicit connections was not tested for in this study. Therefore, although the extent of sewer infrastructure did not change with *E. coli* concentrations, other sewer system factors should not be ruled out as possible contributors to the elevated *E. coli* levels.

Creek Versus Outfall Concentrations

Results indicate *E. coli* levels did not differ between water collected from stormwater outfalls and water collected from the creek. Outfalls tended to have higher *E. coli* levels than the creek, which can be partially explained by the first flush concept. Grab samples were collected in the first few storms of the wet season, which provided ample time for pollutants to build up on the land within the watershed. The first flush concept demonstrates that the highest pollutant levels are often found during the first storm events

as runoff washes pollutants to streams, and levels are lower in subsequent storm events (Lee & Bang, 2000; Qin et al., 2010; Stumpf et al., 2010). Other potential reasons why outfall levels tended to be higher include the possible presence of biofilms in storm drain sewer systems. Biofilms within stormwater sewer systems provide a protected environment for fecal indicator bacteria to replicate, including water and nutrients, and protection from UV light sources, drying, and other microbial predators (Skinner, 2010; Burkhart, 2013). Prior research has also suggested that biofilms can be flushed out of stormwater sewer systems during storms (McCarthy, 2009). Lower *E. coli* concentrations seen in creek samples of this study can be partially attributed to dilution. As outfall water filled with high level bacteria enters the creek, it mixes with larger volumes of water coming from upstream. The mixing of outfall water and creek water thus lowers the overall concentration of bacteria through dilution. In addition to dilution, bacteria can move from the water column and settle to bottom sediments as stream flow decreases, or become resuspended in the water column during high flows (Gao, Falconer, & Lin, 2011). It is possible that *E. coli* settled out of the water column and into streambed sediments, and was not captured in grab samples collected from the water column. In contrast, during an intense storm event, which was not captured in this study, the *E. coli* in streambed sediments could become disturbed enough to resuspend into the water column (Bradshaw et al., 2016).

Residential Versus Industrial Microwatersheds

Creek samples were significantly higher when draining adjacent industrial microwatersheds compared to residential microwatersheds This was interesting as outfall

samples that directly drain these catchments did not find significant or even near significant differences between these land uses, suggesting that in urban reaches of Coyote Creek, microwatershed specific land use types do not change with *E. coli* concentrations directly draining the microwatershed. Although not statistically analyzed in this study, creek samples collected from adjacent open space microwatersheds did have lower means when compared to the urban land uses of residential and industrial. Previous studies have found differences in fecal indicator bacteria between agricultural, open space, and urban land uses (Bolstad & Swank, 1997; Donohue et al., 2006; Dodds & Oakes, 2008); however differences between residential and industrial land uses within urban areas in relation to bacteria in surface water are not well documented. Potential sources of elevated *E. coli* levels found adjacent to industrial areas may be from point sources along creek banks not being drained by the stormwater sewer system. Point sources have been previously documented to weaken common correlations between land use and water quality within a watershed (Zhou et al., 2016).

Encampment Impact

Weak correlations in this study show that bacteria sources could potentially relate to encampments along the creek, but this research did not provide evidence of strong effects. Homeless encampments are prevalent in certain urban stretches of Coyote Creek, noted by the frequency of encampment cleanups conducted by the City of San Jose and Santa Clara Valley Water District from data between 2015 and 2017. The frequency of encampment cleanups in the same locations also suggests encampments may be a continuous source of bacteria to Coyote Creek year-round, but further research is needed

to provide evidence to support this theory. Because encampment frequency along creek banks in San Jose is high, there is minimal buffer area available between the encampments and the creek itself, increasing the potential for fecal bacteria to enter the creek through storm related runoff. Increased buffer lengths have been shown to greatly decrease fecal coliform levels in runoff from pastures fertilized with manure (Lewis et al., 2010). Previous studies have speculated that homeless populations could be possible sources of elevated human fecal indicator bacteria to streams, but their presence was not statistically investigated as an independent variable (Izbicki, Swarzenski, Reich, Rollins, & Holden, 2009; Solo-Gabriele, Wolfert, Desmarais, & Palmer, 2000). The current study does not highlight this source as a major contributor to *E. coli*.

Recommendations

Despite the small sample size of this study, high concentrations of *E. coli* were found in both surface waters and outfalls. Due to the significantly elevated levels of bacteria exiting stormwater outfalls, bacterial load mitigation efforts should focus on stormwater retention and treatment in all microwatersheds as a first priority. Additionally, mitigation efforts should focus on microwatersheds with industrial land uses adjacent to the creek. The levels found in grab samples at outfalls and in the creek greatly exceeded recreational water quality standards for *E. coli*, and are a cause for concern, particularly if the source of the bacteria is human rather than animal fecal matter, as human fecal matter carries a greater risk of spreading diseases to humans (Rose et al., 2001). More advanced source identification such as microbial source tracking is recommended to assess health risks if human fecal contamination is present. Microbial source tracking would be

especially useful for analyzing outfall samples, because the source could be linked to a distinct, small catchment area, rather than a creek sample that has sources coming from multiple locations. Confirmation of human fecal material exiting stormwater outfalls by use of microbial source tracking would provide evidence to support further research into possible sewer infrastructure failures occurring in microwatersheds that drain specific outfalls. In addition to microbial source tracking, other studies investigating bacteria pollution in Coyote Creek are recommended, including conducting similar sample collections and enumeration of bacteria with a much larger sample size; collecting multiple samples from the same site to obtain geometric means for stronger comparison to recreational water quality standards, and further investigation of activities in and surrounding industrial microwatersheds identified along Coyote Creek.

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